PATENT
Attorney Docket No.: 08830-0344US1
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent application of Koen Vandenbroeck *et al.*	Conf. No. 6304
Serial No.:	10/537,647	Group Art Unit: 1636
Filed:	December 28, 2005	Examiner: HIBBERT, Catherine
S		
For:	**RECOMBINANT CELL LINE**	

DECLARATION OF PROFESSOR CARMEN GUAZA UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Professor Carmen Guaza of Instituto Cajal, C/ Doctor Arce 37, 28002 Madrid, Spain, hereby declare and state as follows:

1. I have worked in the field of IL-12 heterodimeric cytokines for 8 years. My particular interest is the establishment of signalling pathways in the regulation of IL-12/IL-23 by endocannabinoids. My *curriculum vitae* is attached hereto as exhibit 1. I am currently the leader of Neuroimmunology Group, a position I have held for 18 years. My duties involve the coordination and direction of research in the group, teaching as well as participation on evaluation committees.

2. I have read and understood US Patent Application 10/537,647 and the claims as presently pending at the United States Patent and Trademark Office (USPTO). I have also read
   - the Office Action issued by the USPTO on the above application dated 24 June 2010,

- Martens *et al.* (Martens E, Alloza I, Scott CJ, Billiau A, Vandenbroeck K., 2000 "Protein disulfide isomerase-mediated cell-free assembly of recombinant interleukin-12 p40 homodimers", Eur J Biochem 267, p 6679-6683),
- Barski *et al.* (US 6,630,324) and
- Graham (Graham L.D. 2002 "Ecdysone-controlled expression of transgenes", Expert Opin Biol Ther, Vol. 2, p525-535), as cited in the Office Action.

3.0 Martens *et al.*

3.1 With respect to the first step of Claim 28, the Examiner alleges, Martens *et al.* "disclose a method comprising incubating a cell culture comprising cells transfected with a baculovirus expression vector comprising DNA encoding the p40 subunit of the dimeric form of IL-12, under the control of an inducible promoter with a compound of interest." (see page 4 of the Office Action).

3.2 Further, Martens *et al* is alleged to teach "inducing transcription of the dimeric p40 IL-12 in the cells of the culture using an inducer". These assertions are indicated to have basis at page 6680, left col., 1, lines 25-30 of Martens *et al.* However, as a person of skill in the art, my understanding is that Martens *et al* discloses two different methods of expressing interleukin 12, neither of which use both an inducer and allow for expression of dimeric IL-12.

3.3 Firstly, Martens *et al* discusses expression of the mosaic His_6- factor-Xa p40 subunit in *E. coli* BL21 (DE3) LysS from an IPTG inducible expression system based on the vector pET3d carrying a T7 promoter (pET3dP40). Expression was induced with 1mM IPTG (Martens *et al.*, page 6680, left column, lines 28 to 20). This resulted in the formation of protein aggregates (inclusion bodies). Accordingly, the p40 proteins produced by this first method are therefore clearly not in the form of the desired p40 homodimers (Martens *et al.*, page 6680, left column). Thus, Martens *et al* does not teach inducing transcription of dimeric p40 in cells in culture.

3.4 In the second method discussed by Martens *et al*, expression of the interleukin 12 p40 subunits is in baclovirus. In the second method, the promoter of the plasmid backbone PAcGP67A driving p40 expression was the strong baculovirus polyhedrin promoter, which is maximally active at the very late stage of infection when the lytic virus is already killing the host cells. The baculovirus RNA polymerase was stimulated by the viral factor VLF-1 (very late factor 1) to drive transcription from the polyhedrin promoter (Mistretta T.A., Guarino L.A. 2005 "Transcriptional Activity of Baculovirus Very Late Factor 1", J Virol 79(3): 1958-1960). The polyhedrin promoter is not an inducible promoter.

3.5 Thus, in contrast to the assertion made in the Office Action, Martens *et al* does not disclose cells transfected with a baclovirus expression vector comprising DNA encoding the p40 subunit of the dimeric form of IL-12 under the control of an inducible promoter with a compound of induction. (emphasis added to show portion missing from teaching).

3.6 With respect to step (ii), the Office Action asserts that Martens *et al* teaches, "inducing transcription of the dimeric p40 IL-12 in the cells of the culture using an inducer". However, as a person of skill in the art, I would clearly understand Martens *et al*, to teach only inducible expression of the p40 subunits in the *E. coli* BL21 (DE3) LysS system using pET3dP40 induced with 1mM IPTG. As set out at point 3.3, induction in this *E. coli* system does not result in dimeric IL-12, but only produces inactive, insoluble protein aggregates (inclusion bodies). In the baclovirus method discussed at point 3.4 above, it is clear no induction of the p40 subunits occurs. Thus, step (ii) of the method is not taught by Martens *et al*.

3.7 In summary, I do not consider that Martens *et al.* discloses an inducible expression system capable of providing dimeric interleukin. Further, Martens *et al* does not teach an inducible expression system wherein dimeric interleukin is secreted.

3.8 Thus, Martens *et al.* cannot teach or suggest a method of screening a candidate compound for the ability to inhibit dimer assembly and secretion of a dimeric form of

interleukin comprising the step of inducing transcription of dimeric interleukin and assaying the cell culture for the presence of said induced secreted interleukin as required by claim 28. As the inducible method outlined at point 3.3 above solely produces insoluble protein aggregates (inclusion bodies) it would be unsuitable for the screening method, as only a non dimeric form of IL-12 is produced. Thus, using an inducible system as taught be Martens *et al*, the method could not examine the ability of a candidate compound to inhibit dimmer assembly and secretion of a dimeric form of interleukin as required by the claim.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

22 Nov 2010 CARMEN GUAZA

(date) (name of declarant)